



## Research Article

# Hormonal and Metabolic Effects of Short-term Energy Imbalance in Obese-Prone as Compared to Obese-Resistant Individuals

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## Abstract

**Background:** While a majority of Americans are overweight, some individuals maintain a healthy weight in spite of fluctuations in energy intake. This study investigates hormonal and metabolic responses to short-term overfeeding and underfeeding in individuals recruited as obese-resistant (OR) or obese-prone (OP) based on self-identification, BMI, and personal/family weight history.

**Methods:** 58 subjects were studied during eucaloric, overfeeding, and underfeeding phases which included a 3-day run-in diet, 1-day intervention diet, and a study day on which a test meal containing 25% of daily energy intake for the intervention diet was provided. Following the test meal, blood was sampled every 30 minutes for hormones and metabolites, and appetite was assessed using visual analog scales.

**Results:** Overfeeding resulted in increased meal responses for insulin, leptin and triglycerides and decreased responses for ghrelin, glucose and free fatty acids (FFA), and underfeeding resulted in decreased insulin, PYY, GLP-1 and triglycerides and increased ghrelin and FFA responses. Ghrelin levels were higher and insulin levels were lower in the OR as compared to the OP, although these effects were attenuated by overfeeding and underfeeding, respectively. Furthermore, there were greater correlations between appetite ratings and appetite-related hormones in the OR.

**Conclusions:** Few studies have assessed hormonal effects of underfeeding, which here was found to result in decreased insulin, PYY and GLP-1, and increased ghrelin. The greater correlations between appetite ratings and appetite-related hormones in the OR suggest that they are more sensitive to short-term energy imbalance and thus may be better able to adjust energy intake accordingly.

**Keywords:** Overfeeding; Underfeeding; Obesity-Prone; Obesity-Resistant

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**Consent:** We confirm that the patients have given their informed consent for the case report to be published.

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## Introduction

Although the health risks of obesity and related metabolic disorders are well-known, the prevalence of obesity continues to increase, with a majority of Americans now overweight or obese (69%) [1]. Periods of energy surplus are likely to occur frequently and contribute substantially to the gradual weight gain seen in most adults. Intra-individual coefficients of variation in daily food intake average up to  $\pm 23\%$  [2] and it has been shown that US adults consume significantly more energy over the weekend than they do during weekdays [3]. Evidence that brief periods of positive energy balance are clinically relevant comes from a study of “holiday weight gain” [4]. In this study many individuals maintained their body weight over the holiday season, while others (largely the obese) tended to have large gains over a short period of time. Perhaps more importantly, weight gained over this brief period of time tended to remain. In order to achieve energy balance, individuals must either increase physical activity in response to these periods, decrease energy intake on other days, or gain weight [5]. Clearly some individuals maintain a healthy weight in spite of fluctuations in energy intake. It is of great interest to determine what factors prevent these “obese resistant” individuals from gaining weight.

In order to assess these potential differences, we compared individuals who were resistant to weight gain (obese-resistant - OR) to other non-obese individuals who were likely to be at risk for weight gain (obese-prone - OP). Previously we found that thin, OR individuals quickly sensed changes in energy balance (short-term overfeeding) with significant decreases in subjective measures of hunger and increases in satiety, and consumption of less energy in the days following a period of overfeeding [6]. In addition, we have recently found that OP

subjects, as defined here, not only have decreased physical activity and down-regulation of fat oxidation at night following overfeeding as compared to OR, but OP also show altered eating behaviors and neuronal responses to food cues [7-9]. It is unclear whether differences in the ability to adapt energy intake to current energy status are related to differences in nutrient sensing by the brain, underlying behavioral differences, or other physiological differences.

However, differences in hormones and metabolites may also affect response to energy imbalance. The relationship between gut hormones and appetite control has increasingly become an area of interest in obesity research [10, 11]. While a number of studies have evaluated gut hormone levels in response to overfeeding [12-15], and others have assessed the response to acute weight loss [16-20], few have specifically assessed the response to acute underfeeding, and none have evaluated individuals with variable propensity to weight gain.

This study was, therefore, designed to investigate the hormonal and metabolic responses to short-term over- and underfeeding in OR and OP individuals. Although weight gain does not result from only one period of overfeeding, the use of a model of short-term energy imbalance allows us to assess hormonal and appetitive responses which are likely to play a role in long-term weight trajectories. We hypothesized that the OR would be more sensitive to changes in levels of appetite-related hormones, with more significant associations between hormone levels and ratings of appetite.

## Methods

**Ethics Statement:** This study was conducted according to the principles expressed in the

Declaration of Helsinki. The study was approved by the Colorado Multiple Institutional Review Board. All patients provided written informed consent for the collection of samples and subsequent analysis.

**Subjects:** Subjects included healthy men and women, ages 25–35 years, without eating disorders or depression, who were empirically classified as either obese-resistant (OR) or obese-prone (OP) as described previously [7-9, 21, 22]. Subjects who were OR had a body mass index (BMI) of 17–25 kg/m<sup>2</sup>, self-reported no first degree relatives with a BMI >30 kg/m<sup>2</sup>, and identified themselves as constitutionally thin based on their perception of difficulty gaining weight despite expending little effort to maintain their current weight. These individuals responded to advertisements asking “Have you always been thin?” and reported no history of ever being overweight. Individuals who were OP, in contrast, responded to the advertisement “Do you struggle with your weight?” They had a BMI of 20-30 kg/m<sup>2</sup>, had at least one first degree relative with a BMI >30 kg/m<sup>2</sup>, reported having to put effort into not gaining weight, and reported previous attempts to lose weight, but were not actively attempting to lose weight. All subjects were weight stable for at least 3 months before being studied and reported that they did not engage in planned physical activity more than 3 hours per week. OR and OP subjects were matched for sex, age (+/- 2 years), and ethnicity/race.

**Study Design and Measurements:** Subjects first underwent baseline assessments, including height, weight and body composition measurement (lean body mass, fat mass, and fat-free mass) by dual-energy x-ray absorptiometry (DEXA) (DPX whole-body scanner, Lunar Radiation Corp., Madison, WI). Each subject participated in 3 study phases separated by at least one month

in a randomized counterbalanced manner, with each phase consisting of a 3 day baseline eucaloric run-in diet period, followed by an intervention diet on day 4, then a study day on day 5. Energy intake during the 3-day run-in diet was tailored to each individual’s needs so as to maintain energy balance, and intake did not differ between study phases for each subject. The three study phases consisted of one of the following on day 4: Eucaloric (EU) diet, Overfeeding (OF) by 40% above estimated energy needs, or Underfeeding (UF) by 40% below baseline caloric intake. During all study periods, the diets were made up of the same macronutrient composition (50% carbohydrate, 30% fat, and 20% protein). Estimates of daily energy needs were made using lean body mass (as determined by DEXA) in the following equation: Resting Metabolic Rate (RMR) = (fat free mass • 23.9) + 372. The estimates were confirmed using RMR as assessed by indirect calorimetry, multiplied by an activity factor of 1.3. This method has been used successfully by our group to maintain energy balance in a number of prior studies [6, 23-27]. All food was prepared and provided by the Clinical Translational Research Center (CTRC) metabolic kitchen. Subjects presented to the CTRC every morning, ate breakfast, and picked up the remainder of their daily meals. Subjects were asked to maintain their usual pattern of physical activity and were regularly questioned regarding activity and compliance. Subjects were asked to not consume any alcoholic or calorie-containing beverages during the study period. In women, study days were scheduled during the follicular phase of their menstrual cycle. In order to assess weight maintenance, all subjects were asked to weigh in on the first day of each study phase and this weight was compared to the weight obtained at their screening visit, and if

the weights differed by more than 3 pounds, the subject would not continue with that study phase. None of the subjects were excluded based on weight changes during the 1 month interval between study phases.

**Study Day:** Subjects presented to the outpatient clinic of the CTRC in the morning after an overnight fast of at least 10 hours. They were weighed and completed subjective appetite ratings measured by visual analog scale (VAS). These included ratings of hunger, prospective food consumption, and satiety. Hunger was rated on a 100-mm line preceded by the question, "How hungry do you feel right now?" and anchored by "not at all hungry" and "extremely hungry" on the right. Satiety was rated by the question, "How full do you feel right now?" with the anchors "not at all" and "extremely" [6]. An intravenous catheter was inserted in a dorsal hand vein for blood sampling. Blood was drawn at baseline for insulin, leptin, glucagon-like peptide-1 (GLP-1), peptide YY (PYY), ghrelin, glucose, free fatty acids (FFA), and triglycerides (TG). Subjects then consumed a liquid breakfast meal within 20 minutes. The energy content of this meal was equal to 25% of the daily energy provided by the intervention diet (EU, OF or UF) and had an identical macronutrient composition. Blood was again sampled at 30, 60, 90, 120, 150 and 180 minutes after initiation of the breakfast meal for insulin, leptin, GLP-1, PYY, ghrelin, glucose, FFA, and TG. Repeat appetite ratings by VAS were also performed 30, 90, 120, 150, and 180 minutes after the meal. The area under the curve (AUC) for all laboratory measures and appetite ratings was calculated using the trapezoid method [28].

**Laboratory Analyses:** Blood samples were collected in EDTA-containing tubes, centrifuged, placed in aliquot tubes and stored

at -70 to -80° C until analysis. All assays were run after all 3 studies phases were complete for each subject. For GLP-1, 30ul of dipeptidyl peptidase IV inhibitor was added to the 4ml EDTA tube prior to collection. GLP-1 assays were performed with Alpco Diagnostics ELISA (43-GPTHU-E01). Insulin concentrations were measured using competitive radioimmunoassay (Millipore). Radioimmunoassay was used to analyze serum leptin (Millipore), serum PYY concentrations (Millipore Cat. #PYYT-66HK) and total serum ghrelin concentrations (Millipore Cat. #GHRT-89HK). All radioimmunoassays were performed with a Perkin Elmer Wallac Gamma counter using Maciel RIA-AID data reduction software. Assays for glucose, TG and FFA were performed on the Olympus AU400e Chemistry Analyzer (Beckman). Reagents were purchased from Beckman Coulter for glucose and TG and from WACO for FFA.

**Statistical Analyses:** Data were analyzed using SigmaPlot version 12 (San Jose, CA). All results are reported as means and standard errors unless otherwise noted. A two-way repeated measures ANOVA was used to examine the effects of overfeeding and underfeeding on all laboratory measures, with p values identified for interactions and main effects of obesity (OP and OR) and study phase (EU, OF and UF). For OP/OR comparisons, both the raw laboratory values and laboratory values corrected for fat mass were used in the analyses. Data are reported for comparisons between the EU phase and OF or UF, but not for OF as compared to UF because these differences are less clinically relevant. For laboratory data with missing time points, if 2 time points or less were missing, then the mean value for the corresponding group and phase was put in for the missing time point. If >2 time points were

missing, the data set for that laboratory value in that phase was thrown out. This occurred for 6 subjects, 5 subjects with only one laboratory value excluded and 1 subject with 2 laboratory values excluded from the analyses. All other values were used in the analysis. GLP-1 results were missing for 10 subjects (4 OR and 6 OP) due to incorrect collection. Correlations were determined using the Pearson product moment formula. All statistical tests were two-tailed with significance set at  $p < 0.05$ .

## Results

**Subjects and baseline characteristics:** A total of 58 subjects were studied, equally divided between male and female (Table 1). OP subjects had greater BMI, fat mass and percent body fat than OR, but lean body mass and fat free mass were not significantly different between groups.

**Table 1 Baseline characteristics.**

	OR	OP
Total n (male/female)	29 (15/14)	29 (14/15)
Age (years)	30.7 $\pm$ 3.4	30.4 $\pm$ 3.9
BMI (kg/m <sup>2</sup> )	20.9 $\pm$ 1.9	26.1 $\pm$ 2.8 <sup>*</sup>
Lean Body Mass (kg)	48.5 $\pm$ 10.3	53.4 $\pm$ 10.4
Fat Mass (kg)	10.7 $\pm$ 3.6	22.7 $\pm$ 8.0 <sup>*</sup>
Percent Body Fat	18.8 $\pm$ 4.6	28.7 $\pm$ 8.0 <sup>*</sup>

Mean  $\pm$  standard deviation for Obese-Resistant (OR) and Obese-Prone (OP)

<sup>\*</sup> $p < 0.001$

**Fasting:** We first report on the effects of OF and UF on fasting levels of hormones and metabolites as summarized in Table 2. These results reflect the effects of the previous day's diet on these variables. One day of OF resulted in an increase in leptin ( $p = 0.002$ ) and a decrease in FFA ( $p = 0.007$ ) as compared to the EU phase, while insulin, glucose, ghrelin, PYY, GLP-1 and TG were unaffected by OF. UF was associated with a decrease in insulin ( $p = 0.032$ ) and PYY ( $p = 0.035$ ) and an increase in FFA ( $p < 0.001$ ) compared to EU. Leptin, ghrelin, GLP-1, glucose and TG, however, were not affected by UF.

**Meal Response:** Next we examine the acute effects of OF and UF on hormones and metabolites over the 3 hours following a test meal (Table 3 and Figure 1). OF resulted in an increase in leptin AUC ( $p < 0.001$ ) and a decrease in ghrelin AUC ( $p < 0.001$ ) compared to EU, but did not affect AUC for PYY or GLP-1. There was an increase in the insulin AUC with OF compared to the EU phase ( $p < 0.001$ ), accompanied by a decrease in glucose AUC ( $p = 0.008$ ). FFA AUC was lower ( $p = 0.002$ ) and TG AUC was higher in the OF ( $p < 0.001$ ) compared to the EU phase.

**Table 2** Fasting values for hormones and metabolites in eucaloric (EU), overfed (OF) and underfed (UF) conditions

	EU	OF	UF
Insulin (ng/mL)	12.9 ± 0.5	13.7 ± 0.5	11.2 ± 0.5 <sup>†</sup>
Leptin (ng/mL)	7.7 ± 0.3	9.1 ± 0.3 <sup>†</sup>	7.2 ± 0.3
Ghrelin (pg/mL)	901.4 ± 12.1	870.9 ± 12.2	892.5 ± 12.1
PYY (pg/mL)	103.3 ± 2.6	100.0 ± 2.6	93.7 ± 2.6 <sup>†</sup>
GLP-1 (pmol/L)	7.5 ± 0.8	7.0 ± 0.8	5.4 ± 0.8
Glucose (mg/dL)	83.8 ± 0.7	85.2 ± 0.7	83.7 ± 0.7
FFA (uEq/L)	525 ± 30	410 ± 30 <sup>†</sup>	671 ± 30 <sup>*</sup>
TG (mg/dL)	86.7 ± 2.8	89.1 ± 2.8	83.1 ± 2.7

Mean ± standard error for all subjects combined

Peptide YY (PYY), Glucose-like peptide (GLP-1), Free fatty acids (FFA), Triglycerides (TG)

<sup>\*</sup>p<0.001 for comparison to EU

<sup>†</sup>p<0.05 for comparison to EU

**Table 3** Area Under Curve (AUC) following a test meal for hormones and metabolites in eucaloric (EU), overfed (OF) and underfed (UF) conditions

	EU	OF	UF
Insulin (ng/mL x 180 min)	8028 ± 314	9927 ± 310 <sup>*</sup>	5303 ± 310 <sup>*</sup>
Leptin (ng/mL x 180 min)	1239 ± 51	1575 ± 50 <sup>*</sup>	1176 ± 9.9
Ghrelin (pg/mL x 180 min)	135921 ± 1589	125483 ± 1569 <sup>*</sup>	142827 ± 1568 <sup>†</sup>
PYY (pg/mL x 180 min)	22045 ± 460	22467 ± 456	19622 ± 454 <sup>*</sup>
GLP-1 (pmol/L x 180 min)	1857 ± 146	1924 ± 143	1374 ± 139 <sup>†</sup>
Glucose (mg/dL x 180 min)	15968 ± 184	15268 ± 182 <sup>†</sup>	15622 ± 182
FFA (uEq/L x 180 min)	38436 ± 1453	32103 ± 1434 <sup>†</sup>	48374 ± 1433 <sup>*</sup>
TG (mg/dL x 180 min)	16582 ± 511	20252 ± 504 <sup>*</sup>	14680 ± 504 <sup>†</sup>

Mean area under curve ± standard error for all subjects combined

Peptide YY (PYY), Glucose-like peptide (GLP-1), Free fatty acids (FFA), Triglycerides (TG)

<sup>\*</sup>p<0.001 for comparison to EU

<sup>†</sup>p<0.05 for comparison to EU



**Table 4** Hormones and metabolites by feeding phase (EU, OF and UF) and by group (OR and OP)

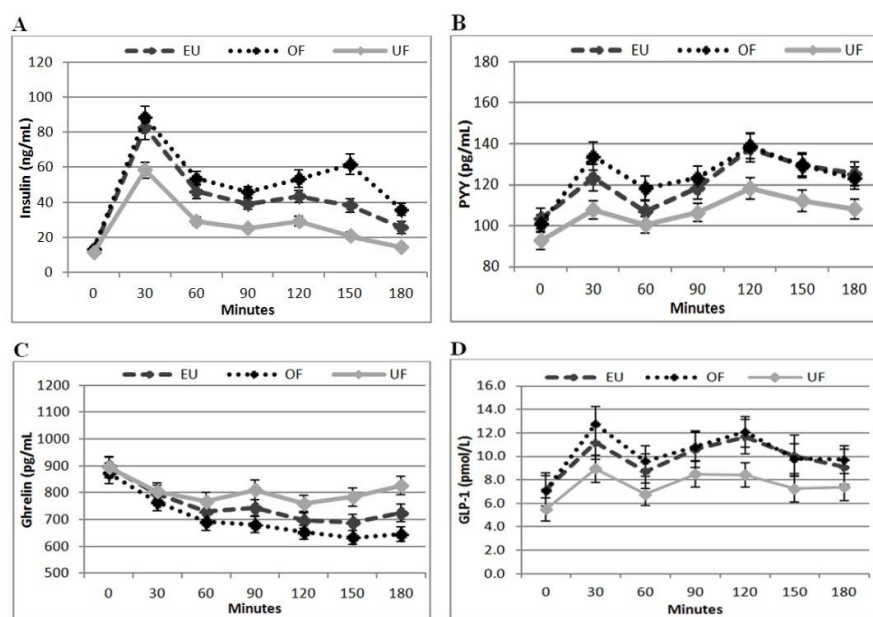
	EU		OF		UF	
AUC	OP	OR	OP	OR	OP	OR
Insulin (ng/mL)	9147 ± 436	6908 ± 452 <sup>†</sup>	10956 ± 448	8899 ± 428 <sup>†</sup>	5695 ± 436	4912 ± 440
Leptin (ng/mL)	1881 ± 72	598 ± 71 <sup>*</sup>	2362 ± 75	789 ± 67 <sup>*</sup>	1826 ± 72	526 ± 69 <sup>*</sup>
Ghrelin (pg/mL)	124586 ± 2207	147256 ± 2286 <sup>†</sup>	116733 ± 2268	134233 ± 2169	130877 ± 2207	154776 ± 2226 <sup>†</sup>
PYY (pg/mL)	21307 ± 658	22784 ± 644	21342 ± 678	23593 ± 610	18518 ± 658	20725 ± 627
GLP-1 (pmol/L)	1713 ± 211	2002 ± 203	1909 ± 217	1939 ± 187	1438 ± 202	1309 ± 192
Glucose (mg/dL)	16332 ± 256	15603 ± 265	15647 ± 263	14889 ± 251	15747 ± 256	15497 ± 258
FFA (uEq/L)	39277 ± 2018	37596 ± 2090	31318 ± 2073	32888 ± 1983	50513 ± 2018	46234 ± 2035
TG (mg/dL)	17250 ± 709	15914 ± 735	20431 ± 729	20073 ± 697	15968 ± 709	13393 ± 715

Mean area under curve (AUC) ± standard error

Peptide YY (PYY), Glucose-like peptide (GLP-1), Free fatty acids (FFA), Triglycerides (TG)

<sup>\*</sup>p<0.001 for comparison to OP

<sup>†</sup>p<0.05 for comparison to OP



**Figure 1** The effects of overfeeding (OF) and underfeeding (UF) as compared to eucaloric feeding (EU) on hormones are shown in response to a test meal: A) Insulin area under the curve (AUC) was increased with OF and decreased with UF; B) Peptide YY (PYY) AUC was decreased with UF but not affected by OF; C) Ghrelin AUC was decreased with OF and increased with UF; and D) Glucagon-like peptide-1 (GLP-1) was decreased with UF but not affected by OF.

UF resulted in an increase in ghrelin AUC compared to EU ( $p=0.002$ ), while PYY AUC ( $p<0.001$ ) and GLP-1 AUC ( $p=0.037$ ) were lower with UF in comparison to EU. Leptin AUC was not affected by UF. UF resulted in lower insulin AUC compared to EU ( $p<0.001$ ), but did not affect glucose AUC. FFA AUC was higher ( $p<0.001$ ) and TG AUC was lower ( $p=0.009$ ) with UF compared to EU.

**Group differences:** Group differences are summarized in Table 4. For all laboratory data, there were no interactions between group and phase. Fasting ghrelin was lower in the OP compared to OR ( $801.0 \pm 49.6$  vs.  $975.5 \pm 49.1$  pg/mL,  $p=0.015$ , mean for all phases), and was significant for all phases individually. Ghrelin AUC (mean for all phases) was lower in the OP compared to the OR ( $124065 \pm 7232$  vs.  $145422 \pm 7227$  pg/mL  $\times$  180 min,  $p=0.041$ ). Ghrelin was lower in the OP in the EU ( $p=0.039$ ) and UF ( $p=0.028$ ) phases, but not the

OF phase. When adjusted for fat mass, the group differences in ghrelin levels remained significant, both across all phases and for each condition individually. Baseline insulin sensitivity as calculated by homeostasis model of insulin resistance (HOMA-IR) did not differ between groups. Although there was no difference in fasting insulin concentrations, there was a trend toward higher insulin AUC (mean for all 3 conditions) in the OP as compared to OR ( $8599 \pm 625$  vs.  $6906 \pm 625$  ng/mL  $\times$  180 min,  $p=0.06$ ). Within phases, insulin AUC was higher in the OP in the OF ( $p=0.047$ ) and EU ( $p=0.05$ ) but not UF conditions. When adjusted for fat mass, the group differences again remained significant across all phases as well as for OF, but not EU or UF. Fasting leptin was also higher in the OP compared to OR ( $12.1 \pm 1.1$  vs.  $3.9 \pm 1.0$  ng/mL,  $p<0.001$ , mean for all phases), as was leptin AUC ( $2023 \pm 181$  vs.  $638 \pm 171$  ng/mL  $\times$  180 min,  $p<0.001$ , mean for all phases). These differences were significant for all 3 phases individually as well. However, the differences



disappeared after adjusting for fat mass. There were no differences between OP and OR for fasting or AUC of PYY, GLP-1, glucose, TG or FFA (mean for all phases or for any condition individually). The difference between AUC for phases (EU-OF and EU-UF) were also examined by group for all laboratory data. There was a greater difference between OF and EU for leptin in the OP as compared to OR, but this difference disappeared after adjusting for fat mass. For all other laboratory analyses, there were no group differences for change in AUC by phase.

**Laboratory measures and appetite ratings:** OF resulted in lower hunger and higher satiety, and UF resulted in higher hunger and lower satiety with no group differences seen [9]. For both groups combined, there was a correlation between insulin AUC and satiety AUC ( $r=0.189$ ,  $p=0.015$ ). Within the OR group, there was a correlation between fasting insulin and pre-meal satiety ratings ( $r=0.279$ ,  $p=0.01$ ) as well as between insulin AUC and satiety AUC ( $r=0.369$ ,  $p<0.001$ ), but insulin and satiety (fasting or AUC) did not correlate within the OP group. There was no correlation between ghrelin and hunger (fasting or AUC) for both groups combined or within groups. A correlation was seen, however, between change in ghrelin and change in hunger in the early post-meal phase (from 0-30 min) in the OR group only ( $r=0.219$ ,  $p=0.045$ ). There were no correlations between leptin and satiety (fasting or AUC) for both groups combined or in either group individually. While there were no correlations between leptin and hunger for both groups combined or in the OP, a correlation was seen between fasting leptin and pre-meal hunger ( $r=-0.239$ ,  $p=0.0287$ ) as well as between leptin AUC and hunger AUC ( $r=-0.216$ ,  $p=0.0482$ ) in the OR group. There were no correlations between PYY and satiety (fasting or AUC) for both groups combined or in either group individually. Correlation was seen between GLP-1 and satiety ratings in both groups combined ( $r=0.152$ ,  $p=0.001$ ), as well as in both the OP ( $r=0.150$ ,  $p=0.0320$ ) and the OR ( $r=0.155$ ,  $p=0.0164$ ) groups individually.

## Discussion

The present study was performed to examine the hormonal and metabolic response to short-term energy imbalance in individuals screened to be resistant to weight gain and obesity (OR) as compared to individuals screened to be prone to weight gain and obesity (OP). These results indicate that short-term overfeeding results in increased insulin, leptin and TG response to a meal, while a decreased response was seen in ghrelin, glucose and FFA. Underfeeding was shown to result in not only decreases in meal response for insulin and TG and increases in ghrelin and FFA but also in decreased response for PYY and GLP-1. While it might be expected that OF and UF would affect the same hormones in the opposite direction, the results of this study do not support that hypothesis. In fact, only ghrelin and insulin are affected by both OF and UF, while leptin is affected only by OF and PYY and GLP-1 are affected only by UF. Between-group comparisons showed that the OR had lower insulin responses (EU and OF phases), and higher ghrelin responses (EU and UF phases). Differences between OR and OP do not explain differences in propensity to gain weight, but greater correlations between appetite ratings and appetite-related hormones were found in the OR as compared to OP individuals, which may suggest that OR individuals are more sensitive to physiological hunger cues during brief periods of energy imbalance.

Other studies have shown that short-term overfeeding results in insulin resistance [29, 30] and increases in leptin levels [31, 32]. However, studies of ghrelin levels in response to overfeeding have had conflicting results, with trials showing increased levels [12], no difference [13, 14], or decreased post-prandial ghrelin levels [15]. As ghrelin is the only known circulating orexigen and has been shown to increase food intake and body weight in both rodents and humans [33, 34], overfeeding would be expected to result in decreased ghrelin to counteract the energy surplus.

While there are many studies of the effects of

weight loss on glycemic parameters and gut hormones, acute and short-term underfeeding has not been studied extensively. Reports of insulin sensitivity following short term underfeeding (1-5 days) have had conflicting results, showing decreased insulin sensitivity [35], no change [36], or an increase in insulin sensitivity [37]. Our results of decreased fasting insulin as well as insulin response to a meal are consistent with an improvement in insulin sensitivity following a period of only one day of underfeeding, although it should be noted that the carbohydrate load consumed with the UF test meal was smaller than that consumed in the other conditions. One other study has specifically examined PYY levels in response to one day of underfeeding in which PYY was also found to be decreased [38]. This finding would be expected since PYY is considered to be a satiety hormone, being low in the fasted state and rising rapidly following a meal [39]. Similarly, GLP-1 levels might be expected to be lower after underfeeding, as circulating GLP-1 levels have also been found to rise after a meal and fall in the fasted state, and have been found to reduce food intake [11]. However, our finding of reduced levels of GLP-1 in the underfed state is novel, as several studies have shown that hypocaloric diets resulting in weight loss do not affect GLP-1 levels [18-20]. Although no studies have examined ghrelin levels in response to short-term underfeeding, studies examining ghrelin levels in response to hypocaloric diets have shown increased ghrelin levels in response to diet-induced weight loss [16, 17]. These findings are consistent with our results showing increased ghrelin levels after one day of underfeeding, and would be expected in response to an energy deficit. While the findings of decreased insulin, PYY, and GLP-1, and increases in ghrelin in response to UF are not unexpected based on known physiological effects of these hormones, these effects have not been previously reported in the literature.

The lower insulin response in the OR group could be explained by the difference in baseline BMI, as lean individuals have repeatedly been shown to be more insulin sensitive [40, 41].

However, the results remained significant after adjusting for fat mass. Likewise, the higher ghrelin levels seen in the OR might be expected since ghrelin levels have been shown to be inversely correlated with adiposity [42], but again the results remained significant after adjusting for fat mass. Thus, the differences in insulin and ghrelin responses here do not appear to be related to baseline differences in fat mass. Moreover, no other studies have investigated the correlation between subjective ratings of appetite and appetite-related hormones in OR as compared to OP individuals. Our findings suggest that the OR individuals are more sensitive to these physiological cues of hunger and satiety, which could in part explain their decreased propensity to gain weight. It is possible that their increased sensitivity to changes in appetite-related hormones allows them to more accurately adjust energy intake following periods of over-nutrition, thus maintaining weight stability while others are likely to gain weight.

There are limitations to this study that should be addressed. While there are inherent problems with classifying individuals as being prone or resistant to obesity before its development, we believe that the most important factor in this categorization is self-identification. Subjects were recruited for this study with the use of advertisements directed at individuals who perceived that they either had a tendency to gain weight or a tendency to remain thin. These groups have been previously studied as defined here, with the hope of determining predictors of weight gain over time [7-9, 21, 22]. Ultimately, however, it will be the longitudinal weight data which is currently being collected that will determine whether or not these categories are valid. The fact that the two groups differed with respect to BMI and fat mass at baseline likely reflects the fact that individuals who report struggling with their weight and who perceive a tendency to gain weight are more likely to have already gained weight during their twenties and early thirties. While the difference in baseline fat mass could be hypothesized to explain the decreased insulin and increased ghrelin responses seen in the OR, we did not find that the differences

were accounted for by fat mass. Moreover, differences in fat mass are unlikely to have played a role in correlations between hormones and appetite ratings. It should also be emphasized that the test meals provided on the study days differed in caloric content based on condition (EU, or 40% above or below for OF and UF respectively) and that this affects interpretation of the results. While the fasting values for hormones and metabolites reflect the state of energy balance created in the previous 24 hours, the meal response values reflect both the fasting levels and the response to a meal of variable caloric content.

## Conclusions

In summary, our results indicate that overfeeding results in increases in insulin and leptin with decreases in ghrelin levels, while underfeeding results in decreases in insulin, PYY and GLP-1, with increases in ghrelin levels. The decrease in PYY and GLP-1 and concomitant increase in ghrelin after underfeeding lend support to the idea that caloric restriction (as employed during dieting) causes changes in gut hormones that promote food intake and might impede weight loss efforts. Moreover, the finding that OR individuals show greater correlations between these hormones and subjective sensations of appetite suggests that these individuals may be more sensitive to energy imbalance and thus better able to adjust energy intake accordingly.

## Abbreviations

OR – obese-resistant; OP – obese-prone; BMI – body mass index; DEXA – dual-energy x-ray absorptiometry; EU – eucaloric; OF – overfed; UF – underfed; RMR – resting metabolic rate; CTRC – clinical translational research center; VAS – visual analog scale; GLP-1 – glucagon-like peptide-1; PYY – peptide YY (PYY), FFA – free fatty acids; TG – triglycerides; AUC – area under the curve; EDTA – Ethylenediaminetetraacetic.

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